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Predicting Single-Species Toxicity in Natural Water Systems

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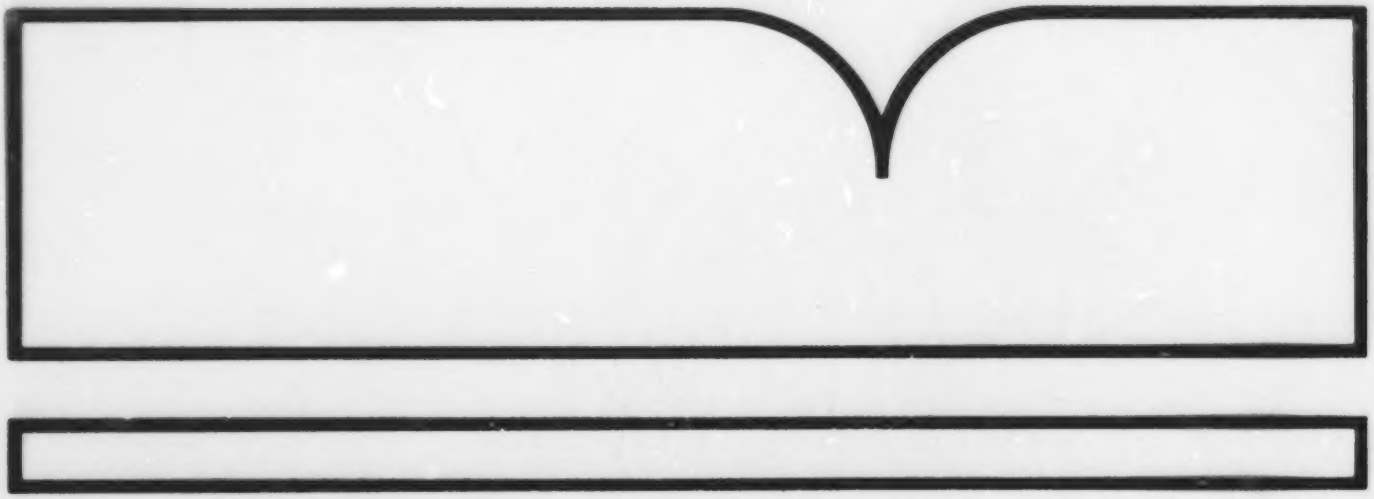

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A methodology is proposed to predict single-species toxicity in natural waters by using laboratory bioassay data which relate effect to a tissue concentration of toxicant or to injury accumulation. Such relationships should be independent of test conditions and therefore transferable from lab to field. A mathematical model of uptake and elimination of toxicants by fish is used to relate exposure concentration to tissue concentration and then to affect. Prediction of toxicity in a laboratory test in which the exposure concentration varies in time is presented as a first step in testing the methodology. Survival time under time-varying exposure is calculated and compared to observed data. The model predicted survival times that were consistent with the observed times. Whole-body residue is used as the estimate of dose.

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Hazard Assessment

PREDICTING SINGLE-SPECIES TOXICITY IN NATURAL WATER SYSTEMS

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Abstract—A methodology is proposed to predict single-species toxicity in natural waters by using laboratory bioassay data which relate effect to a tissue concentration of toxicant or to injury accumulation. Such relationships should be independent of test conditions and therefore be transferable from lab to field. Data for endrin and copper are presented which illustrate relationships between tissue concentrations and mortality. A mathematical model of uptake and elimination of toxicants by fish is used to relate exposure concentration to tissue concentration and then to effect. Prediction of toxicity in a laboratory test in which the exposure concentration varies in time is presented as a first step in testing the methodology. Using a dose–response relationship determined from published data for zinc toxicity to rainbow trout in a constant exposure toxicity test, the survival time under time-varying exposure is calculated and compared to observed data. The model predicted survival times that were consistent with the observed times. Whole-body residue is used as the estimate of dose.

A methodology for considering chemicals for which tissue concentration is not a useful estimate of dose is also presented. Diazinon-induced acetylcholinesterase inhibition data are used to demonstrate this methodology.

Keywords—Toxicity Mathematical model Dose Injury accumulation

INTRODUCTION

The toxicity of chemicals to aquatic life has historically been estimated using the laboratory bioassay test, in which the relationship between a constant concentration of chemical in the water and the response of a test species population is measured[1]. This relationship is a surrogate for the more fundamental dose–response relationship that is the classical basis of toxicology. The exposure concentration is substituted for dose because the rate at which the species takes up chemical from the water is not easily determined.

A primary use of the results of the laboratory bioassay has been in the establishment of water quality criteria for the protection of aquatic life in natural water systems. Because of this use, questions have arisen with regard to extrapolation from the lab to the natural environment [1]. These questions have focused on several issues, two of which are: (1) how to relate the constant exposure in the laboratory to the time variable exposure characteristic of natural water systems, and (2) how to consider the additional exposure to the chemical which occurs in natural water systems through food and sediment.

A third question relates to the validity of extrapolating the rates of chemical uptake and excretion implicitly included in the laboratory toxicity estimate directly to species in the natural environment. A species exposed to a chemical absorbs a portion of that chemical, generally across the gill and/or the gastrointestinal tract. The chemical absorbed by the animal is the dose. Absorbed chemical is transported internally, sequestered at various sites, excreted and possibly metabolized or inactivated. Through some mode of action, the chemical interferes with a bodily function, resulting in the observed toxic effect. The magnitude of the toxic effect is related to the dose and the resulting concentration of the chemical at the site of action. Because the bioassay test directly relates the exposure concentration to the toxic effect, all the intermediate steps must be independent of the conditions of the test if the results are to be extrapolated to a natural system. It may be reasonable to assume that the relationship between internal concentration, mode of action and toxic effect are similar for laboratory and field animals. However, the rates of uptake and excretion-inactivation, and consequently the dose received, may be very different between laboratory and field animals because of variation in metabolic rate with temperature, body weight and activity level.

All three of the questions posed can be ad-

dressed by a laboratory bioassay result that relates internal concentration to response. Such a relationship would be independent of the variability or route of exposure or the rates of uptake and excretion-inactivation. However, additional questions are raised, specifically with regard to the proper measure of internal concentration and the ability to determine its magnitude, in both the laboratory and the natural environment. It is the purpose of this paper to demonstrate, for certain chemicals, a valid measure of internal concentration and present a mathematical modeling framework to compute its magnitude and the associated toxicity in laboratory or field animals. Mortality is used as the measure of toxicity, although the approach is also applicable to sublethal toxicity. The approach addresses single chemicals rather than complex effluents because the toxic agents in complex effluents are generally unknown, precluding a measurement of internal concentrations.

INTERNAL CONCENTRATION AND TOXICITY

The toxic effect of a chemical may be related to the accumulation of chemical at the site of action or to the accumulation of injury. In the former case, the concentration of the chemical may be used as the measure of toxicity without regard to the mode of action, whereas in the latter case a relationship between the chemical and the resulting injury must be established.

Where the concentration of chemical is an appropriate measure of toxicity, a remaining difficulty is the determination of the site of action at which concentration should be measured. However, concentration at the site of action may be proportional to concentration in other body tissues and toxicity may then be related to concentration in those tissues. For example, lake trout fry mortality has been related to the DDT content of eggs [2] and the analysis of fish blood concentration of endrin [3] and parathion [4] indicated threshold values above which fish mortality occurs. In addition, the assumption of a direct relationship between internal concentration and mortality has been used to extrapolate bioassay data for several chemicals to a threshold concentration [5,6] and to predict mortality under time varying exposures in the laboratory [7].

For this class of chemicals, the dose–response relationship may be expressed in terms of the tissue or internal concentration. Figure 1 shows the probability distribution of endrin concentration at death in the gill and blood of spot exposed to two concentrations of endrin in water. A single distribution for each tissue is evident. The fact that these distributions are independent of exposure concentration indicates that gill and blood endrin concentrations are valid measures of toxicity even though neither is the site of toxic action. Similarly, Figure 2 indicates that the gill concentration of copper is significantly related to the mortality of bluegill sunfish. Again, the lack of dependence of the distribution on exposure concentration signifies its validity as a measure of toxicity.

For those chemicals that cause toxicity through the accumulation of injury rather than the accumulation of the chemical, it may be necessary to directly relate injury and response. This approach has been used for organophosphate and carbamate pesticides which cause toxicity by reducing the concentration of the enzyme acetylcholinesterase (AChE). Several studies have correlated brain AChE activity with fish toxicity both in the lab [8,9] and in the field [10,11]. Internal concentration of these pesticides is not the appropriate measure of toxicity because the reduction of AChE caused by the pesticide is only slowly reversible. Continual reduction occurs as additional pesticide is taken up even if that uptake is balanced by the loss of pesticide and there is no concentration increase.

MODELING TOXICITY USING INTERNAL CONCENTRATION

Methodology

For chemicals whose toxicity may be expressed by an internal concentration–response relationship, a prediction of toxicity may be obtained by calculating the internal concentration in animals exposed to the chemical. The concentration in an aquatic organism is the result of several uptake and loss processes that include: transfer across the gills, surface sorption, ingestion of contaminated food, desorption, metabolism, excretion and growth. These processes are controlled by the bioenergetics of the organism and the chemical and physical characteristics of the chemical. The equations to describe these processes in terms of whole-body concentration have been formulated and successfully applied to single species [12,13] and food chains [14–16]. The general equation may be written as:

$$\frac{dv}{dt} = K_u c + \alpha C v_p - (K + G)v \qquad (1)$$

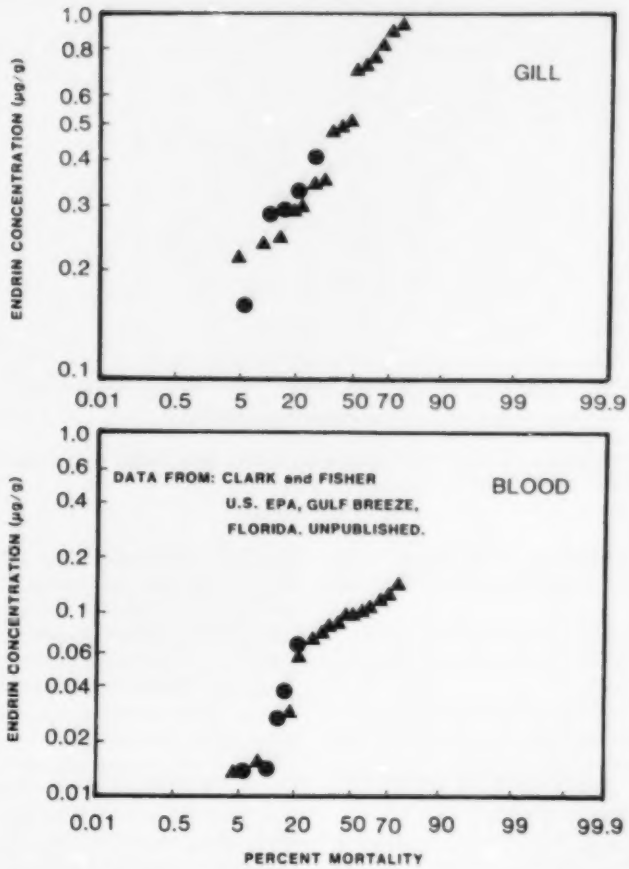

Fig. 1. Probability distribution of endrin concentration at death in the gill and blood of spot exposed to 0.17 µg/L (▲) and 0.31 µg/L(●). Unpublished data of Clark and Fisher. U.S. EPA, Environmental Research Lab, Gulf Breeze, FL.

where v = concentration of chemical in the organism; (µg/g); K_u = uptake rate of chemical from water (L/d-g); c = dissolved chemical concentration (µg/L); α = assimilation efficiency for chemical in food; C = consumption rate (g-prey/g-pred.-d); v_p = concentration of chemical in the prey (µg/g); K = excretion rate of chemical (d^{-1}); G = growth rate of the organism (g/g/d).

For species at the base of the food chain or laboratory species exposed to chemical in water only, Equation 1 reduces to

$$\frac{dv}{dt} = K_u c - (K + G)v \qquad (2)$$

Whole-body concentration calculated from Equation 1 or Equation 2 may be compared to the whole-body concentration–response relationship to determine the toxicity to the species. Alternately, the threshold whole-body concentration that elicits a response may be used with the equations to calculate the maximum allowable exposure concentration. For the estimate of toxicity, internal concentration is calculated in relation to time. The maximum value may then be compared to the internal concentration–response relationship to determine the percentage of the population expected to exhibit the toxic response. Similarly, from the internal concentration–time profile, a

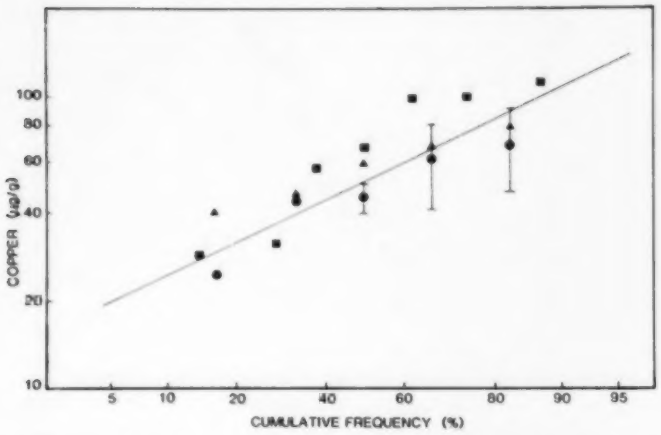

Fig. 2. Probability distribution of copper concentration at death in the gill of bluegill sunfish exposed to 1.56 mg/L(■), 2.52 mg/L(▲) and 7.85 mg/L(●) [24].

relationship between exposure time and percentage of the population exhibiting the toxic response may be developed.

The coefficients in Equations 1 and 2 may be expressed in terms of bioenergetics and incorporate the effects of temperature, activity level and body weight. The exposure concentrations can be functions of time and space. As a result, environmental variability can be incorporated in the toxicity prediction, providing a means of extrapolating from the laboratory to the field.

Application

A laboratory acute toxicity study was used as a preliminary application of the methodology outlined above. A calculated whole-body concentration-mortality relationship from one bioassay test was used with Equation 2 to predict mortality in a second test in which exposure conditions were different.

Figure 3 presents the distribution of survival times for rainbow trout exposed to constant zinc concentrations of 4 mg/L and 6 mg/L [17]. From these data, a whole-body concentration-mortality relationship was calculated using Equation 2. Growth rate in Equation 2 was assumed to be zero and K_u and K were varied to produce a single relationship between cumulative mortality and whole-body zinc concentration from the two survival time distributions (Fig. 4). The values used for K_u and K, 1500 L/kg/d and 1/day, yield a bioconcentration factor of 1500, which is within the range of measured bioconcentration factors for zinc [18]. These values and the relationship between whole-body zinc concentration and mortality provide the necessary information to predict mortality resulting from exposure to the other concentrations of zinc used in the study.

Figure 5 shows two patterns of fluctuating zinc concentration to which the rainbow trout were exposed and the resulting distributions of survival times. For these exposure concentrations, temporal profiles of whole-body zinc concentrations were computed. By noting the times at which computed whole-body concentrations reached values corresponding to specific percentages of population mortality given by the whole-body zinc concentration-mortality relationship, a distribution of survival times was generated for each exposure pattern. These distributions are compared to the observed distributions in Figure 6. For both exposure patterns, the predicted mortality is a reasonable estimate of the observed mortality.

MODELING TOXICITY THROUGH ACCUMULATION OF INJURY

Methodology

In similar fashion to modeling toxicity using internal concentration, those chemicals whose toxicity may be expressed by an injury-response relationship can be modeled by calculating the injury in the animal. This involves the additional step of modeling the relationship between the internal concentration and the injury. The kinetic description for the injury is specific to the type of injury, but may be functionally expressed as a rate of injury

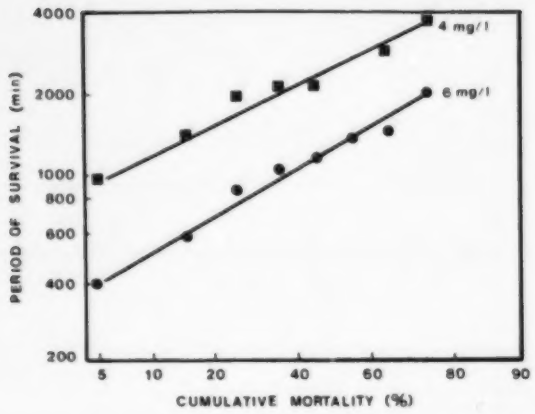

Fig. 3. Distribution of survival times for rainbow trout exposed to constant zinc concentrations of 4 mg/L and 6 mg/L [17].

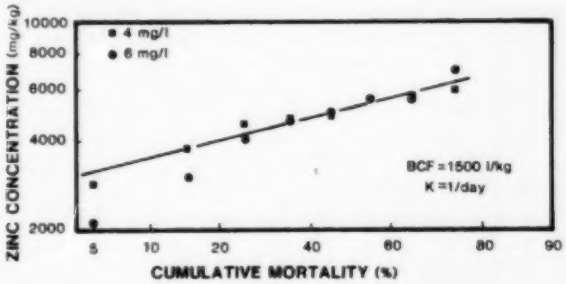

Fig. 4. Calculated whole-body zinc concentration in rainbow trout in relation to cumulative mortality of the test population.

production minus a rate of recovery. The rate of injury production may be proportional to the internal concentration of the chemical and possibly the concentration of the biochemical constituent reacting with the chemical. The rate of recovery may be proportional to the rate of production of new biochemical constituent and possibly the rate of metabolism or excretion of the complex formed by the chemical and the biochemical constituent.

Application

To illustrate the modeling of injury accumulation, the exposure of sheepshead minnows (*Cyprinodon variegatus*) to diazinon is considered. Diazinon is an organophosphate insecticide whose mode of action is a reaction with the enzyme acetylcholinesterase (AChE) to produce a relatively stable phosphorylated enzyme [19]. The resulting reduction of AChE causes excessive accumulation of the neutrotransmitter acetylcholine and death through interference with nerve function.

The reaction sequence of an organophosphate or carbamate pesticide, PX, and AChE, EH, may be written as [19]:

$$EH + PX \underset{K_2}{\overset{K_1}{\rightleftarrows}} EHPX \tag{3}$$

$$EHPX \xrightarrow{K_3} EP + XH \tag{4}$$

$$EP + H_2O \xrightarrow{K_4} EH + POH \tag{5}$$

The first reaction (Eqn. 3) is the formation of a reversible binding complex. This is followed by phosphorylation of the enzyme (Eqn. 4) and then regeneration of the enzyme by dephosphorylation

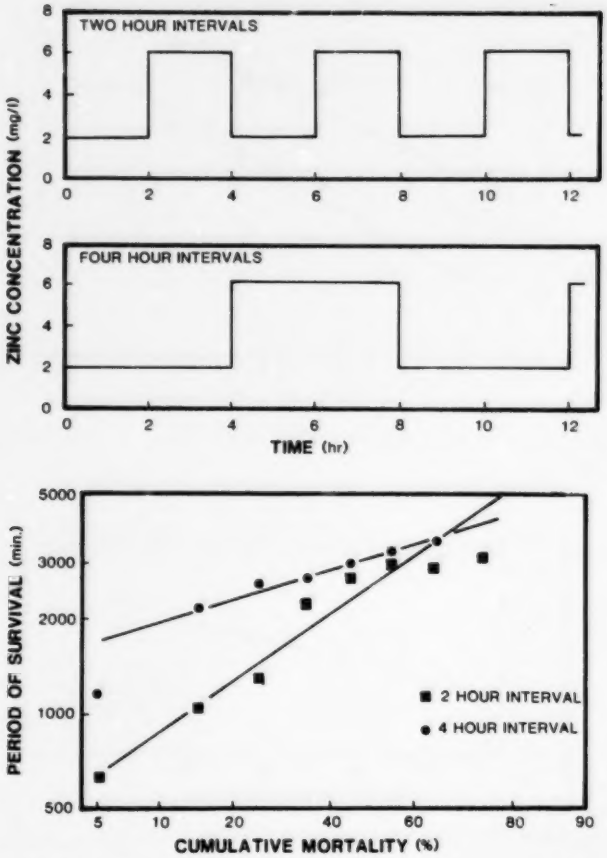

Fig. 5. Zinc exposure concentration pattern and the associated distribution of survival times for rainbow trout [17].

or hydrolysis (Eqn. 5). The rate of phosphorylation, K_3, is usually much higher than the disassociation rate, K_2. In addition, the rate of enzyme regeneration, K_4, is low for organophosphates and in some cases may be insignificant relative to the synthesis of new enzyme by the liver [20]. This is particularly true for diethyl phosphates such as diazinon, which show recovery half-lives in fish in the order of several weeks [21,22].

If the disassociation rate and the enzyme regeneration rate are assumed to be insignificant and the rate of synthesis of new enzyme is assumed to be proportional to the difference between the homeostatic enzyme levels, EH_s, and the current enzyme level, the equation defining AChE may be written as:

$$\frac{d}{dt}[EH] = K_s[EH_s - EH] - K_i'[EH][PX] \quad (6)$$

K_s = rate of synthesis of new AChE (d^{-1}); K_i' = rate of reaction of AChE and the pesticide ($g/\mu g$-d); [EH] = AChE activity (μM acetylcholine hydrolyzed/hr/mg); [PX] = pesticide concentration at the site of action ($\mu g/g$).

The pesticide concentration [PX] may be computed using Equations 1 or 2. Although the concentration computed by these equations and the concentration generally measured are not at the site of action, they are valid if they are proportional to concentration at the site of action. The proportionality is implicitly incorporated in the rate constant K_i'.

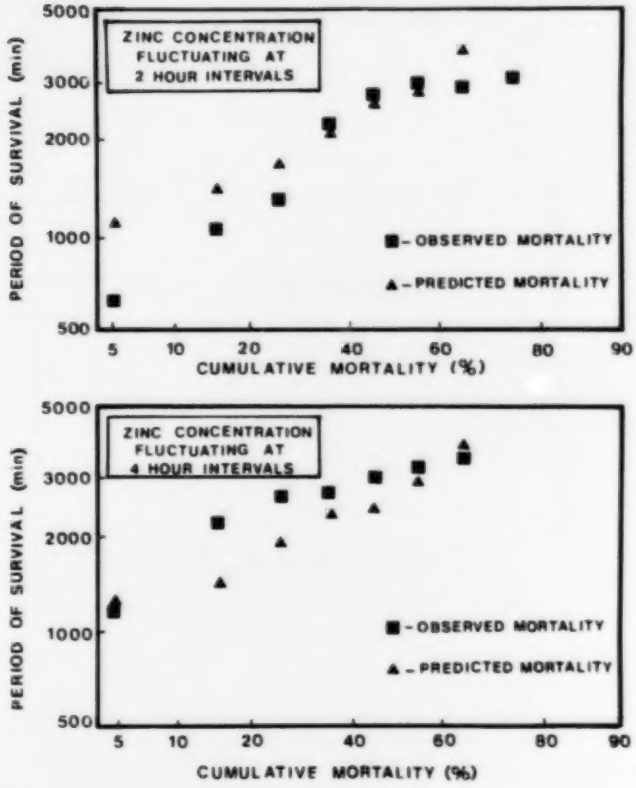

Fig. 6. Observed and predicted distribution of survival times for rainbow trout.

Equations 2 and 6 were applied to a laboratory study [23] of brain AChE inhibition in sheepshead minnows exposed to diazinon. In this study the fish were exposed to a constant concentration of diazinon for a period of 108 d. Brain AChE activity and whole-body (less brain) diazinon residue were measured at several times during this period. Detectable residues were observed for exposure concentrations of 1.8, 3.5 and 6.5 μg/L. Recovery of AChE activity after transfer to diazinon-free water was monitored in fish from the 6.5 μg/L exposure.

Diazinon residues computed by Equation 2 are compared to the observed residues for the three exposure concentrations in Figure 7. The model used a bioconcentration factor of 190 L/kg and a depuration rate of 0.6/d. The computed values are slightly high at 3.5 μg/L and slightly low at 6.5 μg/L, but in general they reproduce the data well.

Equation 2 was then applied simultaneously with Equation 6 to compute AChE activity. Using a homeostatic AChE activity of 1.9 μM acetylcholine hydrolyzed/hr/mg, a synthesis rate, K_s, of 0.2/d, and a reaction rate, K_1', of 0.6 g/μg/d, the model compared well with measured AChE activity at all three exposure concentrations (Fig. 8). The model also accurately computed the recovery of AChE activity.

The ability to compute injury from exposure concentration that is indicated by these results is predicated on the knowledge of the relationship of internal concentration to injury. With that established, the estimation of the effect resulting from a particular exposure situation is dependent on the model of internal concentration. Because this model can incorporate environmental variability, it provides the means of translating a laboratory bioassay to the field. For the example presented

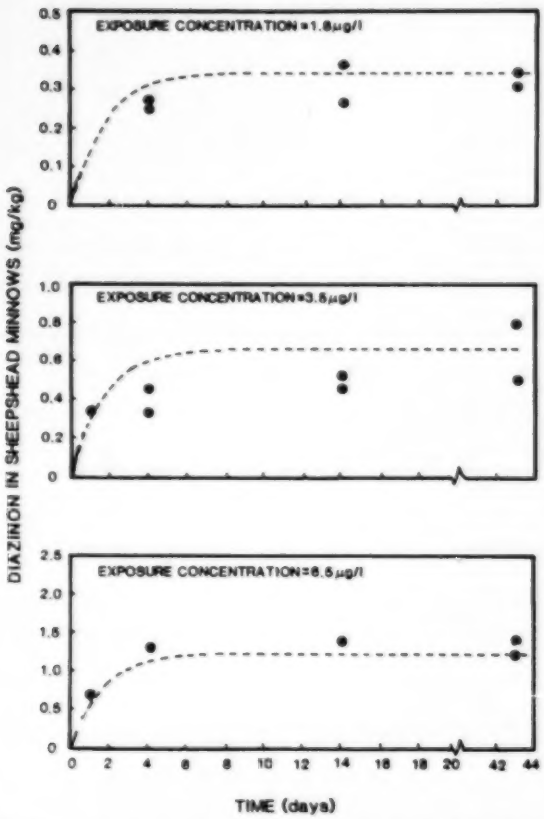

Fig. 7. Observed and computed diazinon concentration in fathead minnows.

here, the necessary bioassay information is the relationship of AChE inhibition to effect. The inhibition calculated by the model may then be used to predict effect for a given exposure situation.

CONCLUSIONS

It is difficult to extrapolate conventional bioassay data to natural water systems. Field exposure conditions differ from those used in the bioassay test and metabolic rates, which control uptake and excretion-inactivation of the chemical, may be different for laboratory and field animals. However, a bioassay test that relates internal concentration or injury accumulation to effect should overcome these problems because it would be independent of exposure conditions and metabolic rates.

Laboratory data presented here demonstrate relationships between mortality and internal concentration whose validity is indicated by their independence of exposure concentration. These relationships also indicate that the internal concentration does not have to be at the site of action of the chemical, a significant observation because concentration may be difficult to measure at the site of action or the site of action may not be known.

Extrapolation of the internal concentration or injury accumulation bioassay involves the calculation of internal concentration, and possibly injury, for the given exposure conditions. Equations previously developed to calculate body burdens in the lab and natural water systems can be used. The utility of the methodology was demonstrated by the successful prediction of zinc toxicity to rainbow trout for differing exposure conditions. The ability to include the additional step of calculating

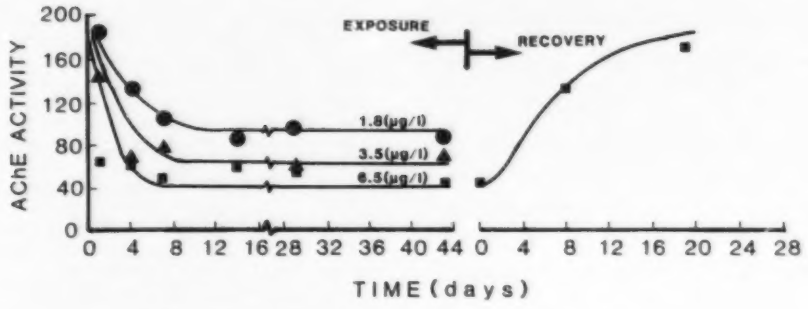

Fig. 8. Observed and computed brain AChE activity in fathead minnows.

injury accumulation was demonstrated by the successful calibration of a proposed AChE inhibition model to observed data.

Further testing of the methodology proposed here is needed before a final assessment of its utility can be made. Applications must include a wide range of chemicals and prediction of toxic effects in the field. In addition, data are needed to test the methodology with those sensitive species, such as *Daphnia magna*, that control hazard assessment. Ultimately, this methodology could be used to estimate the maximum allowable exposure concentration for any exposure condition or the probable toxic effect of a given exposure condition.

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